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Histogenesis Of Neurons In Human Sympathetic Ganglia – A Light Microscopic Study

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Abstract. *The present study was aimed at identifying the various steps occuring in the development of human sympathetic neurons. 90 samples of human fetal sympathetic chains ranging from 8th week to full term were analyzed. Serially cut paraffin sections were stained with hematoxylin and eosine, Cresyl fast violet, Marshland's silver impregnation method and neuron specific enolase immunohistochemical stain. Results of the present study demonstrate that the sympathetic chain in human was laid down in its position dorsilateral to aorta in the trunk region before 8th week of gestation. The chain attained its adult position extending from the cervical level to the sacral level by 12th week of gestation. Histologically the sympathetic chain presented a primitive appearance up to 10th week of gestation. The early sympathoblast stage extended from 12 to 16 weeks. The sympathoblasts were committed to become neurons by 18th week of gestation. The sympathoblasts differentiate to become a neuron by increasing in cell size, accumulating rER and neuronal filaments, putting forth axons and dendrites, and presenting a prominent nucleolus. This process of differentiation took 10 weeks from 18th to 28th week of gestation. Maturation involved a further 6 weeks, from 30th to 36th weeks of gestation, at which time there was an increase in cell size, rER, neuronal filaments and number of dendrites. Only at full term, the neurons attained an individual satellite cell sheath and a clear perineuronal space, which are the characterstic features of adult neurons. The histogenesis of human sympathetic neurons, therefore, involved the following steps, proliferation, migration, commitment, differentiation and maturation. The same pattern and same duration of time was followed throughout the sympathetic chain.*

Key words *: histogenesis; human; sympathetic ganglia; neurons; development; light microscopy*

Introduction :

Neurons of the sympathetic chain belong to the second order neurons in the efferent pathway of the sympathetic part of the autonomic nervous system. These are derived from the multipotent neural crest cells. (*Yntemma and Hammond, 1947; Weston, 1970; Anderson; 1989; Stemple and Anderson, 1992).* These cells migrate ventrally beside the cranial half of the sclerotome, where it forms the dorsal root ganglion *(Leikola 1976)* and migrate further ventralward to form sympathetic chain ganglia *(Goldstein and Kalcheim, 1991).* The migration of the neural crest cells to form sympathetic chain is controlled by the expression of Hox-C genes *(Breier et al, 1988).*

Formation of sympathetic chain is noted around 9mm stage of the human embryo *(Keibel and Mall, 1912)*. Neurons to the human sympathetic chian are contributed by the ventrimedial aspect of the dorsal root ganglion *(Keibel and Mall, 1912; Mitchell, 1953)* and are also derived from the ventral aspect of the neural tube which pass along the ventral nerve roots *(Brizze and Kuntz, 1950).* The same observations were proved in lower animals by cell marking techniques *(Le Douarin and Teillet, 1974; Serbedjiza et al, 1990)*. These are the primitive migratory cells reaching the sympathetic chain initially and depend on insulin like nerve growth factor for their further differentiation *(Le Douarin and Smith, 1988).* Once they reach the sympathetic chain they lose their migratory cytoplasmic processes, get rounded off to form sympathoblasts, the small cells with heterochromatic nucleus *(Page et al, 1986)*. The sympathoblasts differentiate to form neurons and glial cells *(Kiran and Vatsala, 1996)*.

For a neuroblast to differentiate into a neuron, the cells have to follow certain steps. Intially the pleuripotent stem cells should get committed to form neurons *(Norr, 1973; Roufa et al, 1986).* To reach the stage of adult neuron the neuroblast needs to acquire the cytoplasmic organelles, typical of a neuron *(Young and Heath, 2000).* The organelles identifiable under light microscope are the abundance of rough surfaced endoplasmic reticulum called Nissl bodies to manufacture the neurotransmitter substance and neuronal filaments to give shape to the cell and to transport the neurotransmitter substance to the target organs. The neuroblasts need to put forth axon, the first process formation *(Rubin, 1985; Tennyson, 1970)* and further develop dendrites for synaptic contacts. The axons are guided by NCAM along their pathway to reach their target organs which is further supported by the

nerve growth factor elicited by the target organs *(Levi Montalcini and Hamburger, 1951)*. It was shown by immunohistochemical stains in experimental animals that the sympathetic neurons secrete adrenalin at their nerve endings *(Patterson, 1978; Garcia-Arraras et al, 1986, 1992)*. Secretion of neurotransmitter substance at various target organs in experimental animals shows that it ranges from the time of birth to adulthood *(Handa et al, 1993; Yamuchi and Burnstock 1969; Newgreen et al, 1980; Gabella, 1976).*

Most of the above mentioned information was accumulated by cell marking techniques combined with immunohistochemical studies, transgenic experimental studies and Quail-Chick chimera transplantation techniques on experimental animals. Studies on human beings are limited. The present work is designed to investigate : (1) The steps in histogenesis of the sympathetic neurons in the human sympathetic chain, (2) the sequence of steps in the histogenesis of neurons, (3) the time frame for each event in histogenesis, and (4) regional variations if any that occur during this process.

Material and Methods :

17 fetuses from 8th week of gestation to full term were selected from spontaneous abortuses at obstetrics and gynecology department of Sri. Ramachandra Medical College and Research Institute, Deemed University, Porur, Chennai, India, after obtaining necessary permission from the parents. Fifteen of the abortuses belonged to 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36th week of gestation respectively and two foeti were stillborn. The age estimation was obtained from the fetuses' medical records as well as through standard crown rump length (CRL) measurements *(Snell, 1983)*. The fetuses were fixed in formalin. After time percentage the fetuses were opened, organs were eviscerated and the sympathetic chains were dissected from cervical to sacral region. General anatomical features were recorded. A total of 90 samples were taken from the 17 formalin fixed fetuses and were processed for paraffin blocking. In fetuses between 8 & 14 weeks (4 fetuses), samples of cervical, thoracic and lower lumbar region sympathetic chains were processed along with the vertebral column as the chains were too small to be dissected. Therefore, in these fetuses, the specimen included both left and right sympathetic chains. In the remaining 13 fetuses, which were from 16th week to full term, 6 samples from each of the fetuses were obtained. These included right and left superior cervical ganglia (SCG), right and left thoracic chain (THG), right and left lumbar chain (LLG). For the SCG, along with the ganglia a part of the chain was included, while for the THG and LLG, 3 ganglia and the intermittent chain were obtained.

From each fetus, one set of SCG, THG and LLG 6m thick serial sections were cut longtudinally (LS) and another set of SCG, THG and LLG 6m thick serial sections were cut transversely (TS).

Histological Stains

Every alternate section was stained with hematoxylin and eosine (H&E) stain *(Drury and Wallington, 1980).* This stain shows the general morphological features such as the shape, size, cytoplasmic and nuclear details of the neurons. The remaining sections were stained with one of the following stains.

(1) Cresyl fast violet (CFV) is a specific stain to show the rough surfaced endoplasmic reticulum (rER) in the neurons. It imparts a light violet color to the rER. This stain gives a diffused coloration when the rER is less and spread out, and imparts a granular appearance when the rER is abundant *(Young and Heath, 2000)*.

(2) Glees Marshlands silver impregnation method stains the neuronal filaments deep brown. The neuronal filaments are abundant in a mature neuron and their arrangement gives shape to the cell body *(Young and Heath, 2000)*. The neuronal filaments extend parallel to each other.

(3) Neuron specific enolase (NSE) immunohistochemical stain is a major neuronal protein that catalyzes the interconversion of 2 phosphoglycerate and phosphopyruvate. Its immunoreactivity is regarded as an evidence of neuronal cytogenetic differentiation and correlates well with neuronal maturation. Positivity to NSE is used to identify the embryonic cell lineages *(Esiri, 1986)*. The antibody for NSE is supplied by DAKO and the staining procedure is used as specified by DAKO. Chromogen DAB, 3,3 – diaminobenzidine

tetrahydrochloride is used to show the antigen-antibody complex by imparting a brown colour to the end product. Hematoxylin is used as the counter stain to identify the nucleus.

All the sections were analyzed under light microscope and details were recorded.

Results :

GROSS FEATURES

After evisceration of the organs, the sympathetic chains of the embryos between 8th and 14 weeks of gestation were too small to be identified clearly. So, gross features couldn't be recorded. But H & E stained 8th to 10th week sections of cervical, thoracic, lumbar and sacral regions revealed that the sympathetic chain was laid at thoracic and upper lumbar levels, but absent at cervical and sacral levels. By 12th week of gestation sympathetic chains could be identified in cervical and sacral levels as well.

From 16th week onwards, the sympathetic chain was dissected from cervical to sacral region. In the cervical region, except for superior cervical ganglia the remaining ganglia were not distinguishable. However, in the thoracic, lumbar and sacral regions the segmental ganglia with small intermittent chain were visible and with advancing age these features were prominent.

HISTOLOGICAL FEATURES

Cellular morphology

Cellular morphology is best understood in the sections stained with H & E stian. Small cells with heterochromatic nucleus and cytoplasmic extensions were noted between 8th and 10th week of gestaiton (Fig 1). By 12th week of gestation, these cells lost their cytoplasmic extensions, got rounded off and had a heterochromatic nucleus practically filling the whole cell (Fig 2). Between 14th and 16th week of gestation, these uniform rounded cells showed aggregation in a rosette form that consisted of 6 to 12 cells (Fig 3). Rosettes were separated by small spaces. At this stage also, the cells were small with heterochromatic nucleus.

Between 18th and 20th week of gestation, few of the cells in the rosettes showed slight enlargement in size. The cytoplasm appeared intensely stained and the nucleus still remained small and heterochromatic (Fig 4). Between 22nd to 24th week, these cells presented with intensely stained cytoplasm and eccentric, open phased nuclei, Notably, small conical process projecting from one side of the cells was observed, indicative of axon formation. At this stage, these cells were separated by groups of longitudinally oriented cells with oval nucleus. These cells were identified as Schwann cells by S-100 immunohistochemical stain. *(Kiran and Vatsala, 1996)* (Fig 5.)

Between 26th to 28th week of gestation, the cells were markedly increased in size and demonstrated a distinctive irregular appearance. This irregular appearance is evident of formation of dendrites. While the emergence of the dendrites could be identified on the cell body by the spikes, they were too thin to be traced throughout the length of the dendrites. The cells at this stage also showed a big open phase, vesicular eccentric nucleus with a prominent nucleolus. By this time, the immature symphathoblasts had developed to resemble the appearance of mature neurons (Fig. 6).

Between 30th and 36th week of gestation, the cells were big and multipolar in appearance, revealing a vesicular eccentric nucleus with a prominent nucleolus. Two to four cells were enclosed in a (common satellite) cell sheath (Fig 7). These cells were identified as satellite cells by S-100 immunohistochemical stain *(Kiran and Vatsala, 1996).*

By 40th week of gestation, i.e. full term, the cells showed all characteristic features of an adult sympathetic ganglionic neuron along with a separate satellite cell sheath for each neuron and a clear perineuronal space (Fig 8).

Nissl Bodies :

Between 8th to 16th week of gestation rER could not be identified which may be due to the scanty cytoplasm within the cells. Between 18th to 28th week, the cells presented a diffuse Nissl positive cytoplasm, indicating rER to be widely spread (Fig 9). Betwen 30th to 40th week, rER was

abundant and presented granular Nissl positivity (Fig 10).

Neuronal Filaments :

Neuronal filaments could not be identified up to the 22nd week of gestation. From 24th week onwards under Glees Marshlands Silver impregnation method the cells showed a network of dark brown coloured neuronal filaments (Fig 11). With increasing gestational age the condensation of the neuronal filaments increased, which could be observed histologically by an increase in the concentration of the dark brown colouration (Fig 12).

Immunohistochemical study :

NSE stain revealed no distinguishable features in the cells up to the 16th week of gestation. By the 18th week, the cells showed positivity to NSE antibody that could be observed as brown coloration within the cytoplasm (Fig 13). Increased concentration of the dark brown colouration was observed for the NSE protein with advancing gestational age (Fig 14).

The above observations signify the beginning of a particular step as per that gestational age. However, at every stage not all the cells showed the differentiation that has been described. Thus, at any given gestational age, the ganglion revealed maturation pertaining to that age as well as some neurons from the previous stage of differentiation.

A thorough histological study of the sections at cervical, thoracic and lumbar levels revealed the same morphological details in any given gestational age.

Discussion :

Neuronal histogenesis in various parts of the central nervous system has been recorded in detail in recent times (*Shepherd, 1988)* and is spread into the following seven stages : (i) proliferation, during which the cells generate and clone, (ii) commitment to a particular variety of cells like neurons or glia, (iii) cell migration to reach their final destination (iv) differentiation of cells by assuming morphological characteristic features such as cytoplasmic organelles and axon/dendritic extensions, (v) maturation of cells by accumulation and secretion of neurotransmitter substances (vi) myelination, which involved acquisition of myelin sheath and (vii) apoptosis or cell death, involving death of cells that project to abnormal targets and lack the neurotrophic factor. Though above steps are followed in neuronal histogenesis, the exact order of events is variable in different parts of the nervous system.

The human sympathetic chain consists of ganglia and interganglionic cord of the chain. The ganglionic part has sympathetic neurons, which synthesize adrenalin/ noradrenalin enveloped in a satellite cell sheath. The axons of the neurons are guided and myelinated by Schwann Cells. Apart from neurons and glia, the ganglia have other connective tissue including blood vessels. The appearance and maturation of the satellite cells in the developmental aspect of sympathetic ganglia has already been reported by *Kiran and Vatsala (1996)*. The present study deals with appearance and maturation of sympathetic neurons.

Results of the present histological study of the developing human sympathetic neurons were analyzed keeping the above steps of neuronal histogenesis in view. The actual time of occurrence of each step in the development was discussed with the available literature on human embryos and animal models.

Migration :

In the development of the sympathetic chain, it can be said that migration has two phases. The first phase when the neural crest cells migrate to lie dorsilateral to aorta at thoracic and upper lumbar levels and the second phase when it ascends to the cervical and descends to the sacral levels.

A review of the standard textbooks on embryology shows that formation of sympathetic chain is noted by 9mm stage in the human embryos in the thoracic and lumbar regions. *(Datta, 1976; Romanes, 1981; Williams et al, 1995; Snell, 1988)*. Though there is evidence about the exact time of ascent to the cervical region and descent to the sacro coccygeal region in lower forms, (*Gabella, 1976)* the time frame is not available in human embryos. The earliest embryo that could be obtained for the present study was of 8th week of gestation and by this time the sympathetic chain

was already laid at thoracic and upper lumbar regions. So, exact time of migration of the neural crest cells could not be recorded but it could be said that it reached cervical and sacral region only by 12th week of gestation.

At the time of migration the progenitor cells exhibit cytoplasmic processes. It is noted for a long time both by histological studies on human embryos *(Keibel and Mall, 1912)* and even by scanning, electron microscopic studies on avian embryos *(Tonsney, 1988)*. In our study similar cells were noted in the sympathetic chain up to 10th week of gestation. It can be said that migration and primitive stage extended up to 12th week of gestation.

Sympathoblasts :

During development, the primitive cells loose the cytoplasmic processes and get rounded off to form sympathoblasts. Sympathoblasts are the undifferentiated small, rounded cells with hetertochromatic nucleus filling them. *(Page et al, 1986)*. In the present study, this stage with small rounded cells containing heterochromatic nucleus was observed by 12th week of gestation. At this stage there was no ganglionic differentiation. Same small rounded cells, with heterochromatic nucleus were found through out the length of the chain.

Appenzeller (1982) noted rosette formation of the sympathoblasts in 3 months old human sympathetic chains. In the present study, rosette formation, with an aggregation of 6 to 12 cells was noted in 14 and 16th week chains. Still these were small cells with heterochromatic nucleus.

Commitment :

The undifferentiated sympathoblasts get committed by gene expression *(Breier et al, 1988)* and by local environmental factor *(Cohen, 1972; Mizrachi et al, 1990*) to form neuron, Schwann and satellite cells. Commitment to a particular variety of cells can be studied by using immunohistochemical cell lineage technique (*Garcia-Arrasas et al, 1986, 1992)*. NSE antibody is one of the early antibodies developed to identify the cell lineage of neurons by the immunohistochemical stain *(Esiri, 1986; Page, 1986)* in paraffin sections. Presence of NSE shows the commitment of the cell to become a neuron. In the present study positivity to NSE was noted by 18th week of gestation. At this stage only few of the cells in the rosettes show the positivity. Intially it is mild spread of positivity signifying that there is a small amount of neuron specific enolase, which is needed by the cell to function as a neuron. Further there is a gradual incrase of this as gestational age advances.

Differentiation :

Once the premature cell gets committed to form a neuron, it needs to acquire all the characteristic cellular organelles, axons and dendrites to function as a neuron, which in the sympathetic chain is to manufacture and transmit adrenalin or noradrenalin.

Though, a number of animal experimental studies were undertaken *(Garcia-Arrasa et al, 1986; 1992; Cohen, 1972; Wong and Kessler, 1987)* to identify at which stage the phenotypic expression of adrenergic and cholinergic neurotransmitter substances are produced during development, the exact time of chemical coding similar to the adult neurons is not yet fully established.

As such studies are not feasible on human embryos, in the present study an attempt was made to identify the time of histological appearance of organelles like rER, neurofilaments, nucleolus, axons and dendrites and correlate it with their function. The cytoplasmic organelles responsible for the manufacture of neurotransmitter substance is rER. In the present study, it showed that by 18th week the rER in the form of Nissl bodies were noted with Cresyl fast violet stain. It had a diffused positivity at this stage which shows that the Nissl bodies are few and are spread out.

Lot of work has been done by *Rubin (1985), & Tennyson (1970)* on mammalian embryos regarding the appearance of axonal process in development. They showed that the first axonal process appears in the superior cervical ganglion in stage 15 of development. In the present study the axonal process is first seen by 22nd week of gestation. Dendrites also develop simultaneously and they receive the preganglionic fibres from spinal cord at synaptic junction. The synaptic junctions are extensively studied by *Rubin (1985) and Landmesser and Pillar (1974)* who noted that they

are almost 10% at the time of birth and are responsible for the survival of the postganglionic cells. *Purves et al (1986)* have shown that the dendrite formation is seen in rat embryos by 14 weeks of gestation. In the present study, dendritic processes were observed as 2-3 in number by 26th and 28th week of gestation.

Neuronal filaments are the other characteristic organelles needed in a neuron to transmit the neurotransmitter substance and give a shape to the cell *(Heath and Young, 2000).* Prominence of nucleolus is another feature to show the synthesis of neurotransmitter substance. Both these features could be identified in the present study by the 28th week of gestation. Presence of the neuronal filaments was shown by the classical histological silver impregnation techniques.

It can be summarized and said that the cell commitment to form neuron was established by 18th week of gestation. And differentiation extended for 10 weeks from 18th week to 28th week of gestation at which time it has acquired all the cellular organelles and processes required to manufacture and transmit the neurotransmitter substance.

Maturation :

It is generally accepted that neuronal maturation involves cell body maturation with increase in size and accumulation of all cellular organelles, elongation of axons reaching the target organs, development of dendrites, synapses and release of neurotransmitter substances at the target organs. Studies on lower forms *(Yamauchi and Burnstock, 1969; Gabella, 1976; Pattersson, 1978; Newgreen et al, 1980)* have shown that the above features are observed towards end of gestation and continue beyond birth. The present study was limited to histogenesis of sympathetic chain only. Their development near the target organs was not covered in this study. However the stages of cell body maturation are noteworthy.

The neurons between 30 to 36 weeks showed all features of maturation with a gradual increase in cell size, a big vesicular open phase nucleus, a prominent nucleolus, an increase in cell cytoplasm with abundance of granular endoplasmic reticulum, neuronal filaments and an increase in number of dendritic process formation.

Only at 40 weeks, i.e., at the time of birth, the individual neurons attained their adult appearance, which was a fully matured neuron with a complete satellite cell capsule and a clear perineuronal space.

As the present study was limited to the sympathetic chain, the assessment of time of myelination and the appearance of neurotransmitter substance at the target organ were out of bounds.

In summary, histogenesis of the sympathetic chain showed the following pattern (1) Formation & migration; this continued upto 10th week. Intially the sympathetic chain was laid in the thoracic and lumbar regions only. They reached cervical and sacral regions by 12th week of gestation. At this stage the cells presented a primitive appearance with small cell body, heterochromatic nucleus and cytoplasmic processes. (2) Sympathoblasts (12 to 16 weeks): these are immature neuroblasts. They presented two stages. Between 12 and 14 weeks the cells are uniform, rounded cells with heterochromatic nucleus filling the whole cell. By 16th week they are arranged in the form of rosettes with 6 to 12 cell aggregates. (3) Commitment (18 to 28 weeks): by 18th week the neuroblasts were positive to NSE immunohistochemical stain which confirms that these cells are to form neurons. (4) Differentiation: (18 to 28 weeks). By this time the cells have increased in size and showed diffusely spread rER. By 20th week, axon formation is noted. By 24th week the neurons showed neurofilaments, had a prominent nucleolus and presented a multipolar appearance with the formation of dendrites. Cell groups of 2 to 4 cells showed common satellite cell sheath. (5) Maturation (30 to 36 weeks): this showed a general increase in cell size, accumulation of endoplasmic reticulum, granular appearance, dense aggregation of neuronal filaments, inceases in number of dendrites but had a common satellite cell sheath for groups of neurons. (6) Adult appearance (40 weeks): it reached adult appearance by full term with big multipolar cells, heterochromatic nucleus, prominent nucleolus, dense accumulation of endoplasmic reticulum and neurofilaments and a separate satellite cell sheath for each neuron with a clear cut perineuronal space. A thorough histological study of the sections at

cervical, thoracic and lumbar levels revealed the same morphological details in a given gestational age.

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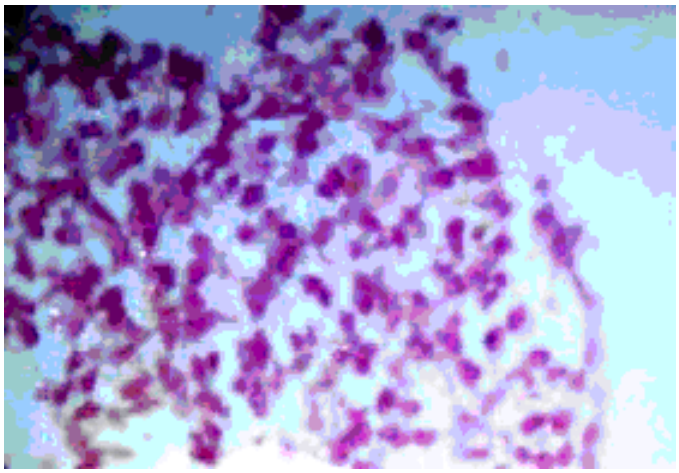

Fig. 1

A TS through LG of 6 cm CRL (10 weeks) fetus showing the primitive appearance of the sympathetic chain with irregular cells having the heterochromatic nuclei. This shows the primitive stage in the development (H & E, 200x).

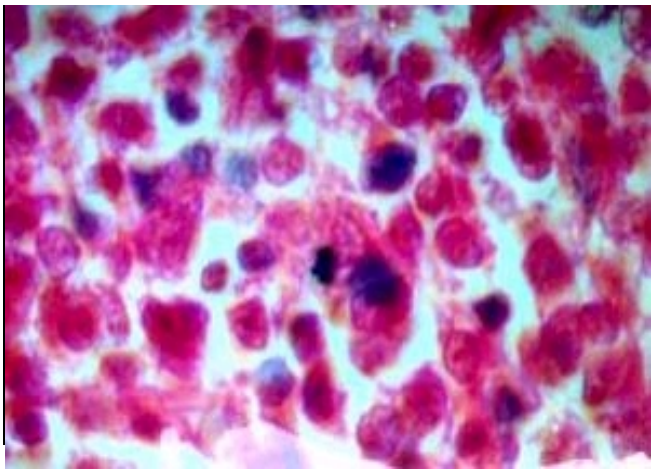

Fig. 2

A TS through THG of 8 cm CRL (12 weeks) fetus showing the small rounded cells with heterochromatic nucleus and scanty cytoplasm. These are identified as sympathoblasts. (H & E, 200x).

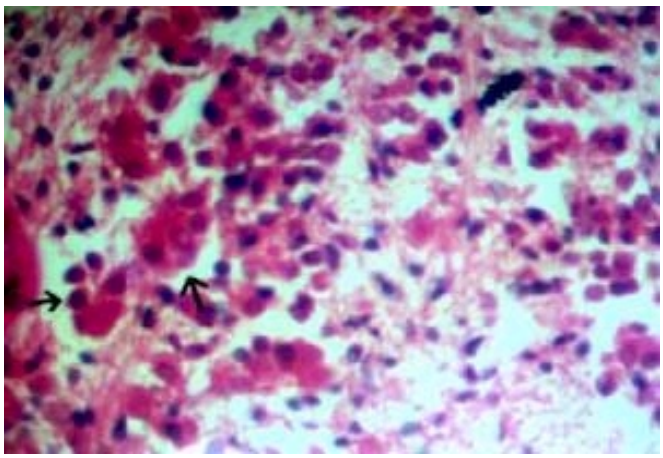

Fig. 3.

A TS through THG of 14 cm CRL (16 weeks) fetus showing the rossette formation of 6 + 2 cells (arrow) (H & E, 200 X).

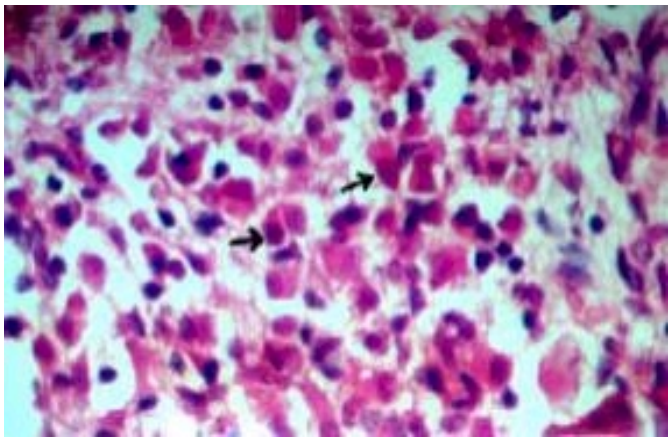

Fig. 4.

A TS through THG of 16 cm (18 weeks) fetus. Most of the neurons show a slight enlargement due to an increase in their cytoplasmic content (arrow). This shows the first step in differentiation (H & E 200x).

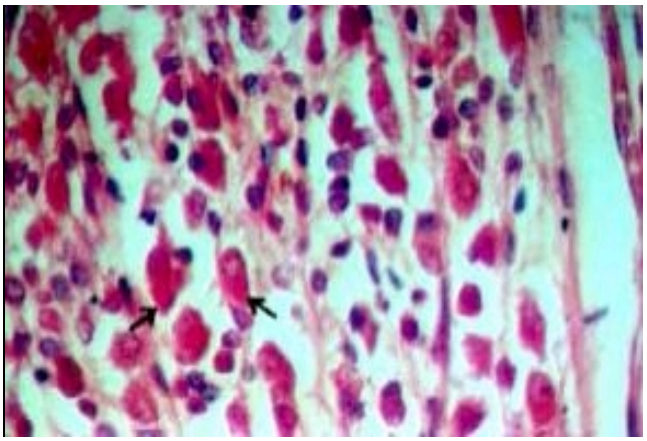

Fig. 5.

A LS through LLG of 22 cm CRL (24 weeks) fetus showing enlarged neurons with conical process (arrows). The stage demonstrates the first step in axon formation (H & E, 200x).

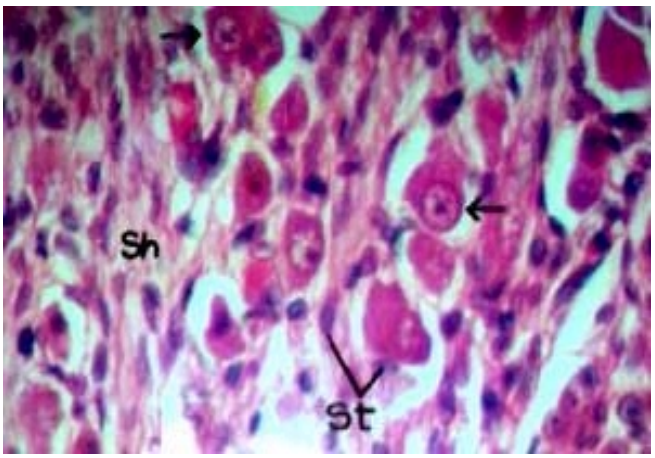

Fig. 6.

A TS through the SCG of 28 cm CRL (28 weeks) fetus. Shows clear multipolar neurons with big open phase nucleus and a prominent nucleolus (arrow). Schwann cells and axons form bundles between the neurons (Sh). Satellite cells cover groups of neurons (St) (H & E, 200x).

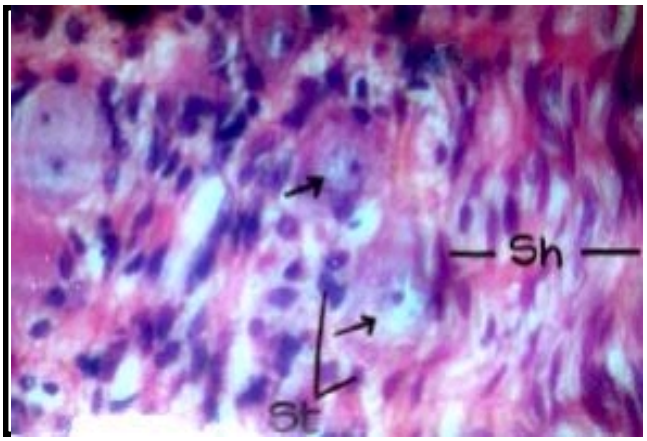

Fig. 7.

A TS of 38cm CRL (full term) fetus. The sympathetic neurons are fully matured. They are seen as single cells (arrow) with a satellite cell sheath (St). Schwann cells are also seen (Sh) (H & E, 200x).

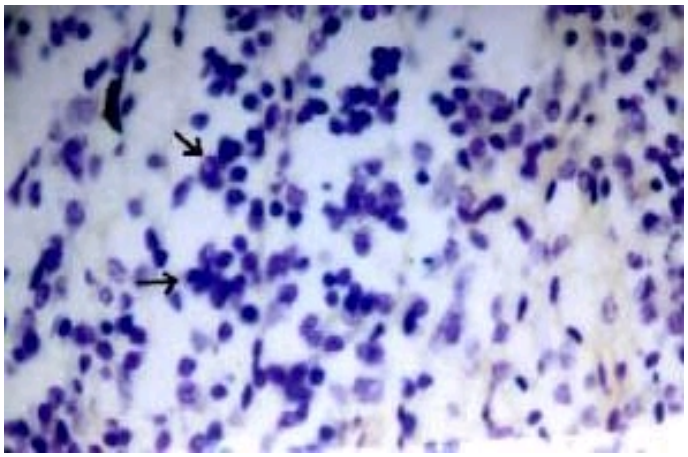

Fig. 8

A TS through THG of 14 cm (16 weeks) fetus. Only nucleus of the cell has taken up the stain (arrow). Cytoplasmic organelles like Nissl bodies have not yet been differentiated (CFV, Mag 200x).

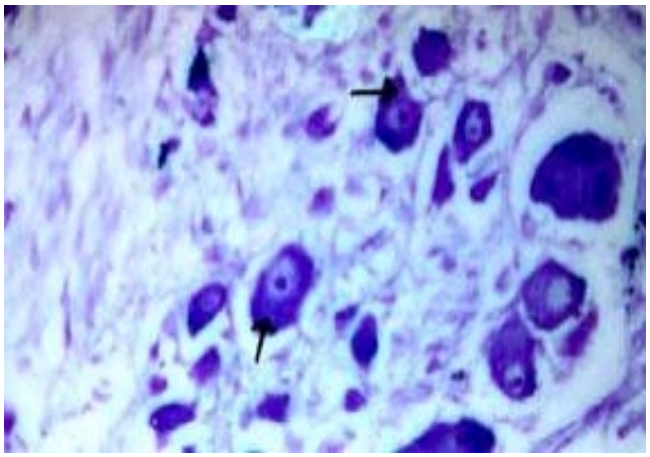

Fig. 9.

A TS through the SCG of 28 cm CRL (28 weeks) fetus. Irregular shaped neurons with open phase vesicular nucleus and diffuse Nissl positive cytoplasm (arrows) are seen. (CFV, mag 200x).

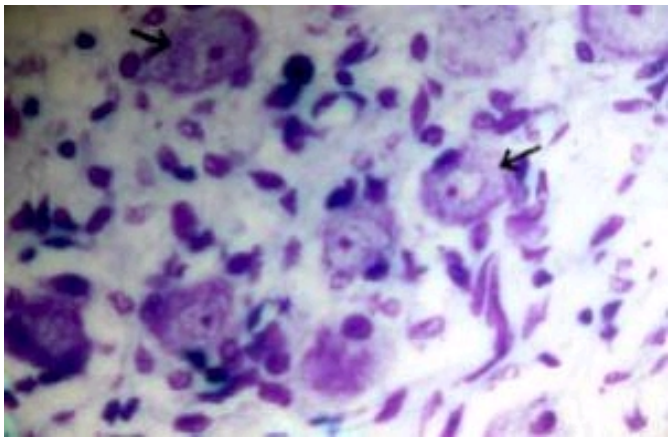

Fig. 10.

A TS through THG of 38 cm CRL (full term) fetus. Shows big hexagonal cells with big vesicular nucleus. Prominent nucleolus and a granular Nissl positive cytoplasm (arrow). Each cell enclosed in a separate satellite cell sheath (St). Schwann cell nuclei are also seen (CFV 200x).

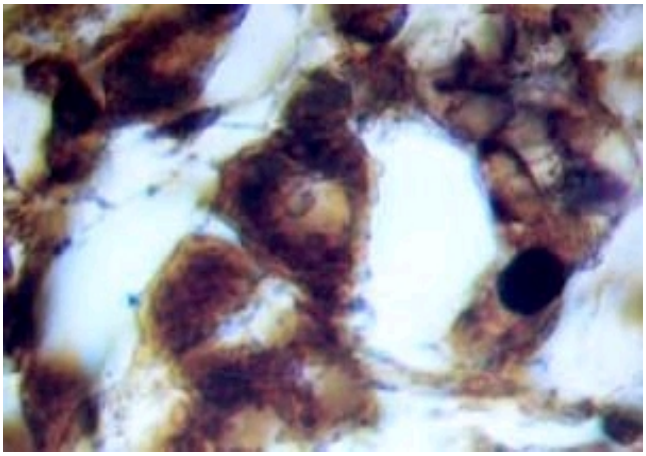

Fig. 11.

A TS through the THG of 28 cm CRL (28 weeks) fetus. A brown coloured spread of neuronal filaments seen in the cytoplasm of neuroblasts. Thin extensions are seen as axons. The nucleus is negative to silver and shows the yellow colour of picric acid (Silver impregnation, 500x).

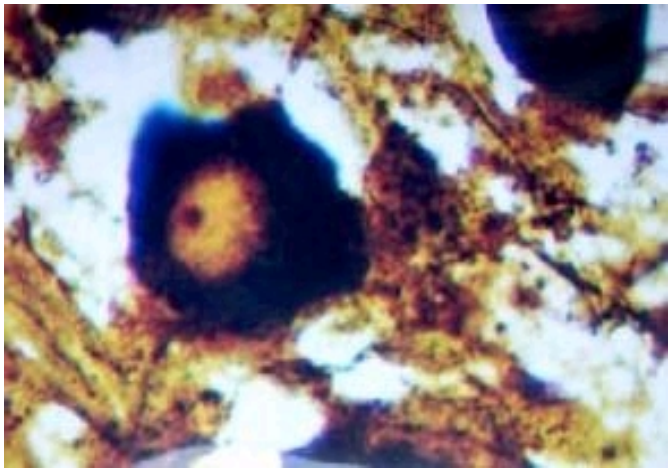

Fig. 12

A TS through the SCG of 38 cm CRL (full term) fetus. Silver impregnation is seen as dark brown coloured concentration that shows the condensation of neuronal filaments filling the cytoplasm of the neurons. The dark brown streaks in between signify the presence of axons and dendrites. Nucleus is negative to silver stain but shows the yellow colouration of the picric acid (silver impregnation, 500x).

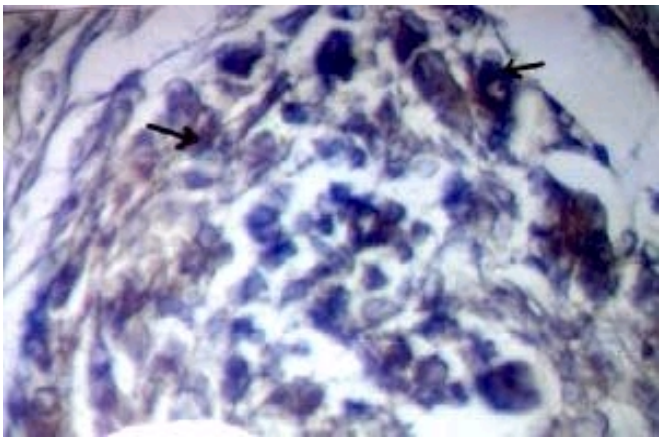

Fig. 13.

A TS through SCG of 16 cm CR (18 weeks) fetus. Shows NSE positive cytoplasm in the developing neurons (arrow) (NSE, 200x).

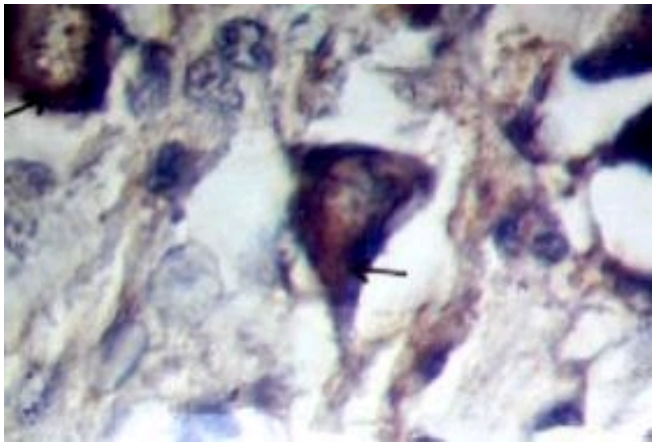

Fig. 14.

A TS through LLG of 34 cm CRL (36 weeks) fetus. Big irregular cells with open phase nucleus and intense NSE positive cytoplasm (arrows) (NSE, 500x).

Literature of human pancreas development are few in number as well as mainly related to first trimester because of ethical and technical difficulties. So the study was conducted on 12 fetuses from 12 gestational weeks (GW) to 5 months of infant to know normal development of exocrine and endocrine part of human pancreas. Material and Methods: Human fetalpancreases were screened by haematoxyline and eosin staining and done electron microscopy for suitable specimens to know ultrastructural detail of fetal pancreas. Results:It was observed arborized tubules, the cells budding out from these tubule